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Fucoidan's conventional and hydrothermal alginate extraction biorefinery: Effect of the extraction of alkaline treatment towards alginate's intrinsic viscosity of brown algae of *Sargassum cristaefolium*

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ABSTRACT

Brown algae can be functioned as the feedstock on the alginate and fucoidan's sequential extraction biorefinery process. This research aims to obtain the condition of the alkaline treatment process, which provides maximum response towards intrinsic viscosity of *Sargassum cristaefolium* alginate on the integrated alginate and fucoidan extraction biorefinery process. Factorial Experiment Design of 2^k was used to understand the effect of temperature, time, and the concentration of Na_2CO_3 towards the response of alginate's intrinsic viscosity and determine the correct equation model on the first order of experiment. The research indicated that the different parameters of temperature, time, and Na_2CO_3 extraction process are tangibly influenced the increasing response of the alginate's intrinsic viscosity, which later reduced after reached its maximum point. The highest alginate intrinsic viscosity of 397.32 ml/g happened during the parameters of the temperature of 60°C, time of 120 minutes, and Na_2CO_3 concentration of 3%. Therefore, the extraction central point design of 60°C temperature, 120 minutes of duration, and 3% concentration of Na_2CO_3 is considered correct, and the first-order polynomial experiment model was shaped in the quadratic form.

Keywords: Biorefinery; alkaline extraction; alginate; intrinsic viscosity; *Sargassum cristaefolium*

INTRODUCTION

Alginate is a polysaccharide in which its component is formed of linear polymers of β -D-mannuronate (M) and α -L-guluronate (G) (Boisseson *et al.*, 2004; Draget and Taylor, 2011). Alginate is available in the cell walls of brown algae, and its amount is around 8-40% (Rahelivao *et al.*, 2013; Sugiono *et al.*, 2019^b). This alginate has no nutritional value, functions as thickener and gel maker, and uses widely in the food and non-food industries (Torres *et al.*, 2007; Hernandez-Carmona, 2013). The availability of alginate is fulfilled from imported products, and its needs are increasing annually. Recently, the demand for alginate is estimated at 2000 tonnes per year (Laksmono *et al.*, 2013). The recent development of domestic alginate extraction method has not found an effective way that can produce high-quality alginate. Therefore, the vast potential of brown algae cannot be used to fulfil the domestic needs of alginate.

The effective alginate extraction method is the integrated alginate extraction based on the industrial biorefinery concept so that the ingredient's utilisation is more optimum and does not produce too many waste products. Brown algae's cell wall also consists of fucoidan, a compound that has a beneficial as natural herbal of anti-oxidant, anti-cancer, and anti-tumour (Ale *et al.*, 2011). Therefore, the integrated alginate and fucoidan's extraction method can be the solution to this problem. The condition of the extraction process and alkaline treatment affects the alginate and fucoidan's quality on the integrated alginate and fucoidan's sequential extraction process (Hernandez-Carmona *et al.* 1999; Lorbeer *et al.* 2015; Silva *et al.* 2015). Therefore, it is essential to study the effect of alkaline treatment towards the alginate's intrinsic viscosity based on the concept of integrated alginate and fucoidan's biorefinery extraction. This research aims to observe the impact of extraction process

parameters of temperature, time, and Na₂CO₃ concentration towards the alginate's intrinsic viscosity of brown algae of *Sargassum cristaefolium*.

METHODS

Materials

Brown algae used for this research is the *Sargassum cristaefolium*, obtained from Poteran island, Sumenep regency. Another chemical materials such as Na₂CO₃, aquades, ethanol 99.8 %, which all have Technical Grade, are obtained from the CV Krida Tama Persada.

Experiment design

Experiment design used in this research is the factorial design of 2^k. Three studied variables are temperature (x₁), time (x₂), and Na₂CO₃ concentration (x₃). These research variables consist of two levels, coded -1 and +1. The centre point is coded 0 with three times of repetition (Gazpersz, 1992). This research's experiment design is shown in Table 1.

Table 1. Factorial design of 2³ and the response of intrinsic viscosity

| No | Actual variable | | | Code variable | | | Intrinsic viscosity (ml/g) |
|----|------------------|---------------|-------------------------------------|---------------|----|----|----------------------------|
| | Temperature (°C) | Time (minute) | Na ₂ CO ₃ (%) | X1 | X2 | X3 | |
| 1 | 90 | 60 | 5 | +1 | 0 | -1 | 122.07 |
| 2 | 90 | 180 | 5 | +1 | +1 | +1 | 66.76 |
| 3 | 90 | 60 | 1 | +1 | -1 | -1 | 151.15 |
| 4 | 30 | 60 | 5 | -1 | -1 | +1 | 160.32 |
| 5 | 30 | 60 | 1 | -1 | -1 | -1 | 152.14 |
| 6 | 90 | 180 | 1 | +1 | +1 | -1 | 93.22 |
| 7 | 30 | 180 | 1 | -1 | +1 | -1 | 132.23 |
| 8 | 30 | 180 | 5 | -1 | +1 | +1 | 147.22 |
| 9 | 60 | 120 | 3 | 0 | 0 | 0 | 392.44 |
| 10 | 60 | 120 | 3 | 0 | 0 | 0 | 382.52 |
| 11 | 60 | 120 | 3 | 0 | 0 | 0 | 397.32 |

Response data of intrinsic viscosity in this research is conducted through regression analysis and model accuracy using the equation of:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \quad (1)$$

In which Y is the response variable, β_0 is the intercept coefficient, β_1 , β_2 , and β_3 are linear regression coefficients, and X₁, X₂, and X₃ are the code of the free variables of temperature, time, and Na₂CO₃ concentration.

Sample preparation

Brown algae are washed by freshwater until clean and later dried under the sun until its water content reached 13%. Dried brown algae later ground by the coffee grinder and filtered by mesh filter No. 60 (Lorbeer *et al.*, 2015). The algae then soaked in the solution of Ethanol 96% and put in the stirrer for one night to eliminate the component of phenol and protein. Afterwards, the algae then washed and dried under the temperature of 45°C for six hours (Ale *et al.*, 2012).

Alginate extraction

Ten grams of brown algae is mixed with Na_2CO_3 solution with the concentrations between 1 to 3 per cent with the solvent ratio of 1:20 (b/v). The alginate later conventionally extracted using the water batch with the temperature between 30 and 90°C with the duration between 60 and 180 minutes (Lorbeer *et al.*, 2015). The processed product later cooled and filtered by the filter press to obtain the A residual and filtrate. Alginate's filtrate then mixed with Ethanol 96% with the ratio of 1:2 (v/v). Afterwards, let it stand for two hours before another filtration stage. The alginate then washed twice using the Ethanol 70% and Ethanol 96% before filtered and pressed. In the end, the alginate dried using the oven of 45°C temperature for as long as 24 hours before grilled by mesh filter No. 60.

Intrinsic viscosity

Obtaining the alginate's viscosity is conducted by measuring using the Ubbelohde capillary viscometer (Canon, USA) with the capillary diameter of 0.56 mm. The measurement was conducted under the temperature of 25°C. Alginate solution was formed by mixing 30 mg of alginate with 10 ml of aquades. The mixture then put into stirrer for 5 hours in the room temperature (25°C). Later, it will be divided into the alginate concentration series between 0.05-0.3 g/dL. Solution's flow time (t) is measured relative to the solvent's flow time (t_0). Intrinsic viscosity then determined using the extrapolation of η_{sp}/c until its concentration reaches zero (Chee *et al.*, 2011).

Relative viscosity, $\eta = \frac{t}{t_0}$ (2)

Specific viscosity, $\eta_{sp} = \eta - 1$ (3)

Reduction viscosity, $\eta_{sp}/c = \frac{t}{t_0} - 1$ (4)

Intrinsic viscosity, $[\eta] = \frac{\eta_{sp}}{c}$ (5)

Data analysis

Data analysis and the accuracy of the polynomial model then statistically analysed using the software of Design Expert version 7.

RESULTS AND DISCUSSIONS

Intrinsic viscosity

The effect of different extraction process parameters of temperature, time, and Na_2CO_3 concentration towards the intrinsic viscosity of *Sargassum cristaefolium* alginate shows that the intrinsic viscosity tends to increase as the temperature rises, times stretches, and concentration thickens. But later, the viscosity decreases after it reaches the maximum point. The alginate's maximum intrinsic viscosity occurred during the temperature of 60°C, the duration of 120 minutes, and the Na_2CO_3 concentration of 3%. The viscosity decreases during the temperature of 90°C, the timestamps of 180 minutes, and the Na_2CO_3 concentration of 5%. The alginate's highest intrinsic viscosity was recorded on 397.32 ml/g. This research result is in accordance with the previous related research conducted by Rahelivao *et al.* (2013), Fenorodaso *et al.* (2010), Fertah, *et al.* (2014), Sellimi *et al.* (2015), and Sugiono *et al.* (2018).

Alkaline treatment extraction process parameters of the temperature of 30-90°C, duration of 60-180 minutes, and Na₂CO₃ concentration of 1-5% are significantly affected (P<0.05) the increase of intrinsic viscosity with the quadratic-shaped pattern. Intrinsic viscosity was rapidly increased in the temperature of 60°C, timestamp of 120 minutes, and Na₂CO₃ concentration of 5%. The viscosity later drastically decreases during the treatment parameters of 90°C temperature, 180-minute duration, and Na₂CO₃ concentration of 5%. This is due to the increase of long-chain polymer alginate extractability on the condition of 60°C temperature, 120-minute duration, and 3% Na₂CO₃ concentration. When the state reached the temperature of 90°C, duration of 180 minutes and Na₂CO₃ concentration of 5%, alginate's intrinsic viscosity drastically decreases due to the degradation of alginate's primary chain polymer. Haug *et al.* (1967) explained that the increase of alkaline concentration during the alginate's extraction process causes the polymer chain degradation due to the β-elimination reaction on the 4-α-glycosidic chain. Polymer degradation of the alginate becomes worse when the pH reaches 12 (Haug *et al.*, 1963; Sugiono *et al.*, 2019^a).

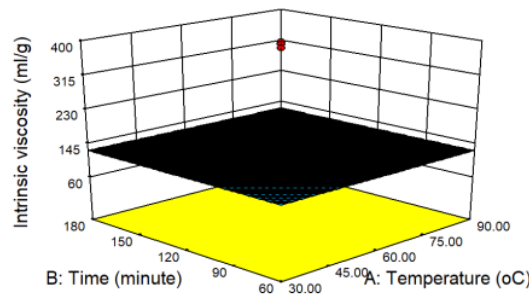


Figure 1. Effects of temperature, time, and Na₂CO₃ concentration towards intrinsic viscosity of *Sargassum cristaefolium* alginate

Model accuracy

The result of the statistical analysis on the intrinsic viscosity of *Sargassum cristaefolium* alginate obtains the first-order experiment polynomial model is as follows:

$$y = 274.30 - 39.68x_1 - 36.56x_2 - 8.09x_3 + 20.06x_1 \cdot x_2 - 19.68x_1 \cdot x_3 + 2.36x_2 \cdot x_3 - 1.05x_1 \cdot x_2 \cdot x_3$$

From the effect of alkaline treatment (temperature, time, and Na₂CO₃ concentration) on the alginate extraction process, it can be concluded that the model is significant on the confidence level of (P<0.05). This result means that the condition during the extraction process is tangibly affected the intrinsic viscosity response. The result of curvature test on Table 2 shows that the curvature model is significant (P<0.05). This means that the intrinsic viscosity of the response model from the experiment's result is formed in quadratic shape (Gazpersz, 1992; Montgomery, 2005).

Table 2. Analysis of first-order polynomial regression varieties

| Variety source | Quadratic sum | df | Middle quadrate | F-value | p-value |
|----------------|---------------|----|-----------------|---------|----------------------|
| Model | 0.000 | 0 | | | |
| Curvature | 1.505E+005 | 1 | 1.505E+005 | 176.82 | 0.0001 |
| Residual | 7659.30 | 9 | 851.03 | | |
| Lack of Fit | 7545.54 | 7 | 1077.93 | 18.95 | 0.0510 ^{ns} |
| Pure error | 113.75 | 2 | 56.88 | | |
| JK Total | 1.581E+005 | 10 | | | |

Note: = Significant
^{ns} = Not significant

The result of this research is in accordance to the report of Sugiono and Ferdiansyah (2018) which stated that first-order experiment polynomial model is quadratic-shaped if the curvature test result is significant ($P < 0.05$). This result proves that the design of the extraction centre point of alkaline treatment on the temperature of 60°C , duration of 120 minutes, and the Na_2CO_3 concentration of 3% is correct.

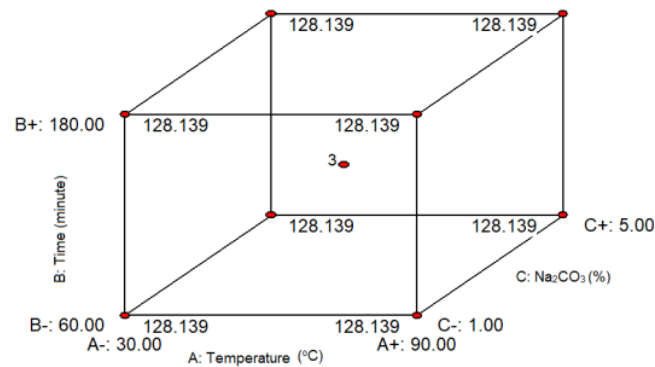


Figure 2. The effects of temperature, time, and Na_2CO_3 concentration towards the intrinsic viscosity of *Sargassum cristaefolium* alginat

CONCLUSIONS

From the research conducted above, it can be concluded that Alkaline extraction treatment (temperature, time, and Na_2CO_3 concentration) provides a significant effect on the intrinsic viscosity of *Sargassum cristaefolium* alginat. This research also found that the highest intrinsic viscosity of 397.32 ml/g occurred during the temperature of 60°C , timestamp of 120 minutes, and Na_2CO_3 concentration of 3%. The first-order polynomial model is formed in quadratic shape, and the centre point designation of 60°C temperature, 120-minute duration, and Na_2CO_3 concentration of 3% is considered correct.

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